Nomenclature of Proteins of Cow's Milk: Fifth Revision[1]

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ABSTRACT

This report reviews changes the nomenclature of bovine milk proteins necessitated by recent advances of our knowledge. Identification of a number of milk proteins (α_{s1}-, β-, and κ-caseins; α-lactalbumin and β-lactoglobulin) continues to be based upon their primary structures (amino acid sequences). Since our last report, α_{s2}-casein and serum albumin can be added to the list of major milk proteins for primary structure is known. Changes recommended in the nomenclature of caseins are primarily a result of differences within this family of proteins brought about by posttranslational modification. For example, α_{s0}-casein is identical to α_{s1}-casein, and α_{s3}-, α_{s4}-, and α_{s6}-caseins are identical to α_{s2}-casein except for differences in degree of phosphorylation. Additionally, proteose-peptone components 5, 8-slow and 8-fast, and γ_1-, γ_2-, and γ_3-caseins are N-terminal and C-terminal fragments, respectively, of β-casein formed during proteolysis by plasmin. Nomenclature of immunoglobulins remains consistent with guidelines for human proteins and is based

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TABLE 1. Proteins of bovine milk and some of their properties.[a]

Protein and suggested abbreviation	Composition in skim milk (g/liter)	Genetic variants	Molecular[c] weights	Sedimentation[d] constant	Isoionic[e] point	Isoelectric[e] point	$A^{1\%}_{1\,cm}$[f]	Hφ[g] avg. (kcal/residue)
α_{s1}-Casein (α_{s1}-CN)	12–15	A	22,068	...	5.15 (243)[h]	...	10.10 (244)	1056
		B	23,614	1.64 (225)	4.92–5.05 (33,233,243)	4.44–4.76 (252)	10.05 (244) 10.0 (258)	1061
		C	23,542	1.64 (225)	5.00–5.35 (233,243)	...	10.03 (244)	1061
		D	23,724	...	...	...	...	1060
		E	23,542	...	...	...	...	1068
					5.16[h] (119)	4.2–4.6[h] (138)	10.7[i], 10.0[h] (9,194)	
α_{s2}-Casein (α_{s2}-CN)	3–4	A	25,230[j]	...	...	...	...	997
		B	...	...	...	...	...	...
		C	...	...	...	...	...	...
		D	...	...	...	...	...	...
β-Casein (β-CN)	9–11[b]	A^1	24,023	1.51, 1.50 (77,195)	5.41 (233)	4.83–5.07 (252)	4.6,4.7 (245,258)	1221
		A^2	23,983	1.51, 1.50 (77,195)	5.30 (233)	...	...	1231
		A^3	23,974	...	5.20 (233)	...	4.7 (245)	1229
		B	24,092	...	5.53 (233)	...	4.5 (245)	1223
		C	23,944	...	5.85 (233)	...	...	1228
		D	23,944	...	...	...	...	1240
		E	23,982	...	...	...		1238
					5.35[h] (119)	4.6–5.1[h] (138)		
κ-Casein (κ-CN)	2–4	A	19,039	...	5.77 (233)	5.45–5.77 (252)	...	1094
		B	19,007	...	6.07 (233)	...	...	1112
				1.4[h] (237)	5.37[h] (119)	4.1[h] (235) 5.3–5.8[h] (138)	10.5[h,i] (91)	
β-Lactoglobulin (β-LG)	2–4	A	18,363	...	5.35 (180)	5.13 (88)	9.6 (248)	1080
		B	18,277	...	5.41 (180)	5.13 (88)	10.0, 9.6[i] (135,248)	1074
		C	18,286	...	5.39 (180)	...	9.5 (248)	1077
		D	18,276	...	...	...	...	1077
		E	18,205	...	...	...	...	1074
		F	18,243	...	...	...	...	...
		G	18,223	...	...	...	...	...
		(Dr)	...	...	...	...		...
				2.7, 1.9[h] (248,249,250)	...	5.14[h] (213)	8.7[i] (24) 10.8[h] (27)	
α-Lactalbumin (α-La)	.6–1.7	A	14,147	...	...	...	...	1016
		B	14,175	...	...	...	...	1022
				1.92[h], 1.98[h], 1.87[h] (156,240,262)		4.2–4.5[h] (156,157)	20.9[h],20.1[h],20.6,20.1[h] (69,156,211,227)	
Serum Albumin (SA)	.4	A	66,267	5.01, 4.0 (15,75)	5.13 (119) 5.15 (222)	4.7–4.9 (213)	6.9, 6.6, 6.49, 6.3 (140,203,215,260)	995

Immunoglobulin G_1 (IgG_1)	.3–.6	153,000–163,000 (28,68,148,164,241)	6.3–7.0 (49,164,241)	. . .	5.5–6.8 (139)	12.1 (241) 13.5 (164)	. . .
Immunoglobulin G_2 (IgG_2)	.05–.1	146,000–154,000 (68,148,164,241)	6.5–7.1 (49,164,241)	. . .	7.5–8.3 (139)	12.1 (241) 12.0 (164)	. . .
Immunoglobulin A[k] (IgA)	.05–.15	385,000–417,000 (50,68,169)	10.8–11.0 (50,68,168,205)	. . .	. . .	. . .	. . .
Immunoglobulin M (IgM)	.05–.1	1,000,000 (188) 960,000 (143)	18.2–19.8 (143,158,168,188)	. . .	. . .	11.8 (241)	. . .
Secretory Component (SC)	.02–.1	79,000	4.0 (50) 4.9 (168)	. . .	. . .	. . .	. . .

[a]Inclusion of values in this Table does not constitute endorsement by the committee.

[b]Values given for β- and γ-caseins were combined and reported as β-CN in this table.

[c]All values, except for immunoglobulins, were calculated based upon known amino acid composition.

[d]Sedimentation constant, S_{20W}, in Svedberg units (1×10^{-13} sec).

[e]See original references for details of methodology.

[f]Absorptivity of a 1% solution measured in a 1 cm light path at 280 nm, except where noted otherwise.

[g]Average hydrophobicity, calculated using free energies of transfer of amino acid side chains from an organic environment to an aqueous environment (30) and known amino acid compositions of the various proteins.

[h]Genetic variant of protein was not reported.

[i]Absorptivity measured at 278 instead of 280 nm.

[j]Molecular weight calculated for α_{S2}-CN 11 P.

[k]All physical chemical data obtained with secretory IgA.

largely upon crossreactivity with reference proteins.

The minor whey protein lactollin is β_2-microglobulin for which the sequence of amino acids is known. An operational definition for proteins associated with the milk fat globule membrane has been developed. Nomenclature initially suggested for these proteins was based upon their electrophoretic behavior under a given set of conditions.

Because of increased interest in milk proteins of species other than bovine, the Committee suggests that these be identified as homologs of those already characterized in European, *Bos taurus*, and Indian, *Bos indicus*, cattle. Guidelines are given to aid in determining if homology exists. Provisional nomenclature is suggested for use in the interim until homology can be established.

INTRODUCTION

The initial report of the American Dairy Science Association Committee on the Nomenclature, Classification, and Methodology of Milk Proteins (129) was an attempt to clarify the nomenclature of milk proteins by "presenting a summary of preferred usage and by showing the relationship between the individual proteins which have been isolated and the classical fractions." Subsequently, this Committee has published a revision of milk protein nomenclature approximately every 5 yr (48, 218, 246, 267) to summarize more recent findings (Table 1) and to suggest changes in nomenclature where appropriate. The intent of this Committee is to suggest a flexible nomenclature system that allows for incorporation of new discoveries rather than to suggest prematurely a rigid system of nomenclature. Since the last report of this Committee (267), the most significant findings related to nomenclature are: determination of primary sequences of α_{s2}-casein and serum albumin, demonstration that members of the α_{s1}- and α_{s2}-casein families (and possibly κ-casein) differ in degree of phosphorylation, determination of the source of several minor proteins in milk as fragments of β-casein produced by plasmin, and identification of lactollin as β_2-microglobulin and determination of its primary sequence. Suggested changes in the nomenclature of the major proteins allow for differentiation based upon differences resulting from posttranslational modifications.

For the first time this Committee recommends an operational nomenclature for proteins associated with the milk fat globule membrane as well as a system, based upon electrophoretic behavior, for identifying individual components. Although many of the proteins associated with the milk fat globule membrane possess enzymatic activity (4, 45, 196), we have elected not to list them, which is in keeping with past practices of not elaborating on enzymes associated with skim milk. These enzymes have been reviewed elsewhere (228).

With the exception of lactollin, we have made no mention of a number of minor milk proteins reviewed by Groves (106) and have followed the pattern previously established (48, 129, 218, 246, 265) in limiting the scope of this report. More detailed treatments on specific aspects of the milk proteins are available in several recent reviews (46, 47, 55, 83, 84, 163, 185, 226, 266). The present report breaks tradition with previous ones by suggesting nomenclature for milk proteins isolated from species other than *Bos*. Because of the increased interest in milk proteins of other species and the tendency of researchers to name these proteins after their counterparts in *Bos*, we felt a responsibility to establish criteria for naming these proteins and determining their homology with bovine milk proteins. At the same time, we respect the prerogative of any investigator to assign nomenclature to any protein with no counterpart in the milks of European, *Bos taurus*, and Indian, *Bos indicus*, cattle.

THE CASEINS

Caseins in milk of the genus *Bos* were defined originally by this Committee (129) as those phosphoproteins that precipitate from raw skim milk by acidification to pH 4.6 at 20°C. In a subsequent report (267), the Committee differentiated caseins according to their relative electrophoretic mobility in alkaline polyacrylamide or starch gels containing urea with or without mercaptoethanol. Based upon current knowledge, we now recommend that use of electrophoresis as a basis for classification be dropped and that caseins be identified according to homology of their primary structures (amino acid sequences) into the following families: α_{s1}-, α_{s2}-, β-, and κ-caseins. Individual members of these families still can be identified by gel electrophoretic techniques, some of the more effective of which are suggested in the monograph by this Committee (238).

α_{s1}-Caseins

The α_{s1}-caseins (α_{s1}-CN) consist of one major and one minor component, both with the same amino acid sequence established by Mercier et al. (187) and Grosclaude et al. (103) for the major component. At present, the five genetic variants are designated A, D, B, C, and E in order of decreasing relative electrophoretic mobilities in alkaline gels containing urea (100, 199, 242). The B variant is predominant in *Bos taurus*, and C is in *Bos indicus* and *Bos grunniens* (99, 242). Bell et al. (23) observed an electrophoretic band in patterns of skim milk protein from Bali (Banteng) cattle, *Bos javanicus*, with slower mobility than the C variant. However, more information is needed before this band can be designated as a unique genetic variant of α_{s1}-CN.

The minor component, previously classified as α_{s0}-casein (267), has the same amino acid sequence as α_{s1}-CN but contains one additional phosphorylated serine residue at position 41 (174, 175). We recommend that variations, which result from posttranslational modification, such as phosphorylation, be designated by an Arabic number and a letter after the Latin letter that designates the genetic variant. In this case, the letter P will indicate that the posttranslational variation is a result of differences in amounts of phosphorylation (31, 32, 100), and the Arabic number will indicate the number of phosphates attached. Thus, the major genetic variant in *Bos taurus* would be designated α_{s1}-CN B-8P instead of α_{s1}-CN B, and the term α_{s0}-casein B would be dropped in favor of α_{s1}-CN B-9P. Because the two α_{s1}-CN components differ only in degree of phosphorylation, genetic variants of both components will be identical in a specific milk. When these proteins are prepared from milk of heterozygous animals in which the particular casein genes show no dominance, two genetic variants will be present.

The primary structure of α_{s1}-CN B-8P is in Figure 1. It consists of 199 amino acid residues: Asp_7, Asn_8, Thr_5, Ser_8, Ser P_8, Glu_{24}, Gln_{15}, Pro_{17}, Gly_9, Ala_9, Val_{11}, Met_5, Ile_{11}, Leu_{17}, Tyr_{10}, Phe_8, Lys_{14}, His_5, Trp_2, and Arg_6 with

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                                                      10                   14                                    20
H.Arg - Pro - Lys - His - Pro - Ile - Lys - His - Gln - Gly - Leu - Pro - Gln - [Glu - Val - Leu - Asn - Glu - Asn - Leu -
                                                                                 Absent in Variant A

                              26                      30                                                          40
[Leu - Arg - Phe - Phe - Val - Ala] - Pro - Phe - Pro - Gln - Val - Phe - Gly - Lys - Glu - Lys - Val - Asn - Glu - Leu -

41                                                    50                53                               59    60
Ser - Lys - Asp - Ile - Gly - Ser - Glu - Ser - Thr - Glu - Asp - Gln - [Ala] - Met - Glu - Asp - Ile - Lys - [Gln] - Met -
[P] (αs1-CN B-9P)             P           P                             ThrP (Variant D)                  Lys (Variant E)
                                                      70                                                          80
Glu - Ala - Glu - Ser - Ile - Ser - Ser - Ser - Glu - Glu - Ile - Val - Pro - Asn - Ser - Val - Glu - Gln - Lys - His -
                  P           P     P     P                                       P
                                                      90                                                         100
Ile - Gln - Lys - Glu - Asp - Val - Pro - Ser - Glu - Arg - Tyr - Leu - Gly - Tyr - Leu - Glu - Gln - Leu - Leu - Arg -

                                                     110                                                         120
Leu - Lys - Lys - Tyr - Lys - Val - Pro - Gln - Leu - Glu - Ile - Val - Pro - Asn - Ser - Ala - Glu - Glu - Arg - Leu -
                                                                                  P
                                                     130                                                         140
His - Ser - Met - Lys - Glu - Gly - Ile - His - Ala - Gln - Gln - Lys - Glu - Pro - Met - Ile - Gly - Val - Asn - Gln -

                                                     150                                                         160
Glu - Leu - Ala - Tyr - Phe - Tyr - Pro - Glu - Leu - Phe - Arg - Gln - Phe - Tyr - Gln - Leu - Asp - Ala - Tyr - Pro -

                                                     170                                                         180
Ser - Gly - Ala - Trp - Tyr - Tyr - Val - Pro - Leu - Gly - Thr - Gln - Tyr - Thr - Asp - Ala - Pro - Ser - Phe - Ser -

                                                     190         192                                       199
Asp - Ile - Pro - Asn - Pro - Ile - Gly - Ser - Glu - Asn - Ser - [Glu] - Lys - Thr - Thr - Met - Pro - Leu - Trp.OH
                                                                  Gly   (Variants C & E)
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Figure 1. Primary structure of *Bos* α_{s1}-CN B-8P (103, 187). The enclosed amino acid residues are sites corresponding to mutational differences in the genetic variants: A, C, D, and E (100, 102). The enclosed P represents the site of additional phosphorylation in α_{s1}-CN B-9P.

a calculated molecular weight of 23,614 (103, 187). The other genetic variants differ from B, as indicated in Figure 1 and Table 2. For variant D, the appropriate nomenclature should be α_{s1}-CN D-9P for the major component and α_{s1}-CN D-10P for the minor (Figure 1, Table 2).

Peptides released during proteolysis of α_{s1}-CN by plasmin have identical gel electrophoretic mobilities under alkaline conditions in the presence of urea as peptides extracted from casein with N,N-dimethylformamide (3). These extracted peptides have complex electrophoretic patterns identical with λ-casein (76), an ill-defined fraction first prepared by Long et al. (165). Two of the peptides produced by plasmin degradation of α_{s1}-CN had tryptic peptide maps and molecular weights identical with two peptides from the N,N-dimethylformamide extract with corresponding electrophoretic mobilities. Although the fraction once identified as λ-casein by this Committee (246) now appears to consist of fragments of α_{s1}-CN, much more definitive work is needed before additional changes are made in the nomenclature.

TABLE 2. Location of amino acid substitutions in genetic variants of milk proteins identified in *Bos*.

Protein	Variant	Position								
		14–26			53			59		192
α_{s1}-CN	A	Deleted								
	B				Ala			Gln		Glu
	C									Gly
	D				ThrP					
	E							Lys		Gly
		18	35		36	37		67	106	122
β-CN	A^1							His		
	A^2	SerP	SerP		Glu	Glu		Pro	His	Ser
	A^3								Gln	
	B							His		Arg
	C		Ser			Lys		His		
	D	Lys								
	E				Lys					
					136	148				
κ-CN	A				Thr	Asp				
	B				Ile	Ala				
						10				
α-LA	A					Gln				
	B					Arg				
		45	50	59	64	78	118	129	130	158
β-LG	A				Asp		Val			
	B	Glu	Pro	Gln	Gly	Ile	Ala	Asp	Asp	Glu
	C			His						
	D	Gln								
	E (D_{yak})									Gly
	F		(Ser)[a]					(Tyr)[b]	(Tyr)[b]	(Gly)[a]
	G					(Met)[a]				(Gly)[a]

[a]Amino acid substitutions placed in parentheses have not yet been established by sequencing the genetic variant.

[b]Tyr substitution for Asp occurs at either 129 or 130.

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                                                            10                                                              20
H.Lys - Asn - Thr - Met - Glu - His - Val - Ser - Ser - Ser - Glu - Glu - Ser - Ile - Ile - Ser - Gln - Glu - Thr - Tyr -
                                            P     P     P                             P
                                                            30                                                              40
Lys - Gln - Glu - Lys - Asn - Met - Ala - Ile - Asn - Pro - Ser - Lys - Glu - Asn - Leu - Cys - Ser - Thr - Phe - Cys -

                                                            50    51    52                                    58    59    60
Lys - Glu - Val - Val - Arg - Asn - Ala - Asn - Glu - [Glu - [Glu - [Tyr - Ser - Ile - Gly - Ser - Ser - Ser] - Glu] - Glu] -
                                                                   (Absent in Variant D)      P     P     P
                                                            70                                                              80
Ser - Ala - Glu - Val - Ala - Thr - Glu - Glu - Val - Lys - Ile - Thr - Val - Asp - Asp - Lys - His - Tyr - Gln - Lys -
P
                                                            90                                                             100
Ala - Leu - Asn - Glu - Ile - Asn - Glu - Phe - Tyr - Gln - Lys - Phe - Pro - Gln - Tyr - Leu - Gln - Tyr - Leu - Tyr -

                                                           110                                                             120
Gln - Gly - Pro - Ile - Val - Leu - Asn - Pro - Trp - Asp - Gln - Val - Lys - Arg - Asn - Ala - Val - Pro - Ile - Thr -

                                                           130                                                             140
Pro - Thr - Leu - Asn - Arg - Glu - Gln - Leu - Ser - Thr - Ser - Glu - Glu - Asn - Ser - Lys - Lys - Thr - Val - Asp -
                                                P           P
                                                           150                                                             160
Met - Glu - Ser - Thr - Glu - Val - Phe - Thr - Lys - Lys - Thr - Lys - Leu - Thr - Glu - Glu - Glu - Lys - Asn - Arg -
            P
                                                           170                                                             180
Leu - Asn - Phe - Leu - Lys - Lys - Ile - Ser - Gln - Arg - Tyr - Gln - Lys - Phe - Ala - Leu - Pro - Gln - Tyr - Leu -

                                                           190                                                             200
Lys - Thr - Val - Tyr - Gln - His - Gln - Lys - Ala - Met - Lys - Pro - Trp - Ile - Gln - Pro - Lys - Thr - Lys - Val -

                                          207
Ile - Pro - Tyr - Val - Arg - Tyr - Leu.OH
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Figure 2. Primary structure (41, 42) of *Bos* α_{s2}-CN A-11P. The amino acid sequences enclosed in brackets represent the possible amino acid sequence missing in the D variant. Only 11 of the phosphorylated serine residues have been tentatively identified (42) and have been designated with a P.

α_{s2}-Caseins

Since the last report of this Committee (267) the primary structure of α_{s2}-casein (α_{s2}-CN) (Figure 2) has been determined (41, 42). It consists of 207 amino acid residues: Asp_4, Asn_{14}, Thr_{15}, Ser_6, Ser P_{11}, Glu_{25}, Gln_{15}, Pro_{10}, Gly_2, Ala_8, Cys_2, Val_{14}, Met_4, Ile_{11}, Leu_{13}, Tyr_{12}, Phe_6, Lys_{24}, His_3, Trp_2, and Arg_6 with a calculated molecular weight of 25,230 (42, 97). Other milk protein components, previously classified as α_{s3}-, α_{s4}-, and α_{s5}-casein by this Committee (218, 267) and α_{s6}-casein by Annan and Manson (9), appear to be components of the α_{s2}-CN family. Evidence strongly suggests that all have the same amino-acid sequence but differ in phosphate content (41, 42). The exact location of the phosphate moieties in each member of the family of α_{s2}-CN has not been determined. Those positions shown as phosphorylated (Figure 2) occur in at least one of the α_{s2}-CN fractions (42). We, therefore, tentatively recommend, until further information is available as to their degree of phosphorylation, that the nomenclature of the A variant, for example, be changed as shown in Table 3. The α_{s5}-casein is a dimer consisting of α_{s3}- and α_{s4}-caseins, now called α_{s2}-CN A-12P and α_{s2}-CN A-11P, linked together by a disulfide bond (120). Until location of this disulfide bond is shown to be unique to these particular fractions, we recommend deletion of this term.

The four recognized genetic variants of α_{s2}-CN are designated A, B, C, and D. The A and D variants have been observed in European breeds (*Bos taurus*) with D in the Vosgienne and Montbéliarde breeds (97). The electrophoretic mobilities of the various D variant bands are slower than the corresponding bands in the A variant at pH 8.6 but are faster at pH 3.0 (97, 98). In addition to variant A, variant B is found in *Bos taurus* and *Bos indicus* in a high Nepalese valley, and variant C is observed specifically in yaks (*Bos grunniens*) in the same region (101). The C variant was also found in yaks from

TABLE 3. Summary of changes recommended for the nomenclature of the α_{s2}-CN A family.

Former nomenclature	Recommended nomenclature
α_{s2}-CN A	α_{s2}-CN A-13P
α_{s3}-CN	α_{s2}-CN A-12P
α_{s4}-CN	α_{s2}-CN A-11P
α_{s6}-CN	α_{s2}-CN A-10P

the Repulic of Mongolia and the B variant in zebu from the Republic of South Africa (98).

Differences among primary sequences of polypeptide chains in genetic variants of α_{S2}-CN, and exact locations and, perhaps, even number of phosphate groups in the various components still remain to be established. Grosclaude et al. (101) compared amino acid and phosphate contents of the C variant with α_{S2}-CN A-10P (Table 4) and postulate a possible substitution of glycine for a phosphorylated serine. Variant D, like α_{S1}-casein A, has a peptide deleted (97, 98); nine amino acid residues are missing from the sequence of α_{S2}-CN A-11P. The exact peptide has not been established because of similarities of the three possibilities: from residues 50 to 58 inclusive, from residues 51 to 59, or from residues 52 to 60 (Figure 2). Because this peptide contains three phosphorylated serine residues, the D variant component corresponding to a α_{S2}-CN A-11P should be named α_{S2}-CN D-8P.

β-Caseins

The primary structure of β-casein (β-CN) (Figure 3) has been elucidated by Ribadeau-Dumas and coworkers (103, 216). Based on this structure, Groves et al. (95, 109, 110) showed that those proteins previously called γ_1-, γ_2-, and γ_3-caseins (267) are actually fragments of β-CN consisting of residues 29-209, 106-209, and 108-209, respectively. Plasmin, a protease in milk (73, 141) is responsible for proteolysis of β-CN, leading to formation of γ_1-, γ_2-, and γ_3-caseins (71). The N-terminal fragments of β-CN released during plasmin degradation also have been identified in milk. Andrews (7, 8) showed that proteins previously called proteose peptone components 5 and 8-fast (267) are identical with fragments of β-CN. Proteose peptone 8-fast consists of residues 1-28, and component 5 is actually the two fragments comprising residues 1-105 and 1-107. Fragments released during proteolysis of

TABLE 4. Comparison of the amino acid and phosphate content of comparable components[a] of variant A and C of α_{S2}-CN (101).

Amino acid	Variants A (residues/molecule)	Variants C (residues/molecule)
Aspartic acid	18	18
Threonine	15	15
Serine	17	11[b,c]
Proline	10	13[c]
Glutamic acid	40	40
Glycine	2	3[c]
Alananine	8	8
Valine	14	14
Methionine	4	4
Isoleucine	11	11
Leucine	13	14[c]
Tyrosine	12	12
Phenylalanine	6	6
Tryptophane	2	...
Lysine	24	24
Histidine	3	3
Argenine	6	6
Cystine	2	...
H_2PO_4	10	8[c]

[a]Component previously designated α_{S6}-CN.

[b]Low value may be an artifact of acid hydrolysis.

[c]Different in amino acid or phosphate content from A variant.

β-CN with plasmin are identical to proteose peptone component 5 (72). The remaining fragments of β-CN (residues 29-105 and 29-107) released during plasmin degradation are proteose peptone component 8-slow (74). Whether proteolysis of β-CN by plasmin occurs primarily in mammary epithelial cells, during temporary storage in the alveolar and glandular lumina, during refrigerated storage, or during protein isolation and purification is not known. Nevertheless, on the basis of these advances in our knowledge, we recommend that fragments resulting from proteolytic cleavage be named as derivatives of the parent polypeptide from which they were derived. A summary of the new recommended nomenclature for the β-CN family is in Table 5.

Seven genetic variants of β-CN are known, but their differentiation by gel electrophoresis is more complicated than for the other caseins. In alkaline gels containing urea (149), they migrate in the order $A^1=A^2=A^3>B>D,E>C$. However, under acidic conditions (149), their order is $C>B=D>A^1=E>A^2>A^3$. Thus, although A variants can be differentiated from B, C, D, and E by electrophoresis under alkaline con-

TABLE 5. Changes recommended in nomenclature of the β-CN family.

Former nomenclature (267)	Recommended nomenclature	Source of fragment
β-Casein A^1	β-CN A^1-5P	...
β-Casein A^2	β-CN A^2-5P	...
β-Casein A^3	β-CN A^3-5P	...
β-Casein B	β-CN B-5P	...
β-Casein C	β-CN C-4P	...
β-Casein D	β-CN D-4P	...
β-Casein E	β-CN E-5P	...
γ_1-Casein A^1	β-CN A^1-1P (f29-209)	β-CN A^1-5P
γ_1-Casein A^2	β-CN A^2-1P (f29-209)	β-CN A^2-5P
γ_1-Casein A^3	β-CN A^3-1P (f29-209)	β-CN A^3-5P
γ_1-Casein B	β-CN-B-1P (f29-209)	β-CN B-5P
γ_2-Casein A^2	β-CN A^2 (f106-209)	β-CN A^1-5P or β-CN A^2-5P
γ_2-Casein A^3	β-CN A^3 (f106-209)	β-CN A^3-5P
γ_2-Casein B	β-CN B (f106-209)	β-CN B-5P
γ_3-Casein A	β-CN A (f108-209)	β-CN A^1-5P, β-CN A^2-5P or β-CN A^3-5P
γ_3-Casein B	β-CN B (f108-209)	β-CN B
Proteose peptone[a] component 5	β-CN-5P (f1-105)	β-CN
Proteose peptone[a] component 5	β-CN-5P (f1-107)	β-CN
Proteose peptone[a] component 8-slow	β-CN-1P (f29-105)	β-CN
Proteose peptone[a] component 8-slow	β-CN-1P (f29-107)	β-CN
Proteose peptone[a] component 8-fast	β-CN-4P (f1-28)	β-CN

[a]These fragments have not been isolated from specific genetic variants. When their genetic origins and degree of phosphorylation are established, appropriate letters and numerals should be employed.

ditions, acidic conditions are required for differentiation of A variants. An additional electrophoretic band with mobility slower than β-CN A^3 under acidic conditions has been observed in the milk of Bali (Banteng) cattle, *Bos* (Bibos) *javanicus* (23). Although this electrophoretic band has been called β-CN A^4, additional evidence is needed prior to actual assignment of nomenclature. In 1968, Aschaffenburg et al. (14) observed a variant of β-CN in Indian and African zebu cattle (*Bos indicus*) that had the same electrophoretic behavior as the B variant but appeared to have different peptide maps. This Committee previously designated this variant B_Z (218). However, Grosclaude et al. (99) observed that the amino acid substitution in the A^1 variant was the same for *Bos indicus* and *Bos taurus* and that the B variant in Choa zebu cattle differed from the A^1 variant by substitution of an arginine residue for a serine residue at position 122, the same difference observed in *Bos taurus*. This raises a question of the existence of a variant B_Z. More work is needed before existence of this variant can be confirmed. The A variants are the predominant polymorphs in all species of *Bos* investigated (242).

The β-CN A^2-5P consists of a single polypeptide chain containing 209 residues (Figure 3): Asp_4, Asn_5, Thr_9, Ser_{11}, Ser P_5, Glu_{18}, Gln_{21}, Pro_{35}, Gly_5, Ala_5, Val_{19}, Met_6, Ile_{10}, Leu_{22}, Tyr_4, Phe_9, Lys_{11}, His_5, Trp_1, and Arg_4 with a calculated molecular weight of 23,983. The other genetic variants differ (99, 102, 104) as in Figure 2 and Table 2. Again, both C and D variants have one less phosphate group than others.

Fragments (f 29-209), (f 106-209), and (f 108-209) have been detected and identified only in β-CN variants A^1, A^2, A^3, and B (Table 5) (95, 109). Examination of milks containing the C variant indicates that the (f 29-209) fragment is missing. The charge reversal occurring in this variant at residue 37 may be related to this phenomenon. While it is expected that these fragments also would occur in variants D and E, they have not been reported specifically. Both β-CN A^1-5P and A^2-5P yield the same fragment (f 106-209) because the point of genetic mutation occurs prior to residue 106. Therefore, this fragment is called β-CN A^2 (f 106-209) to provide a definitive nomenclature. Similarly, β-CN A^1-5P, A^2-5P, and A^3-5P yield the same (f 108-209) fragment, which by

```
                                                   10                                                   18          20
H.Arg - Glu - Leu - Glu - Glu - Leu - Asn - Val - Pro - Gly - Glu - Ile - Val - Glu - Ser - Leu - Ser - Ser - Ser - Glu -
                                                                                     P           P     P     P
                                                                                                      Lys (Variant D)
                                   28 ↓ 29    30                            35    36    37                      40
Glu - Ser - Ile - Thr - Arg - Ile - Asn - Lys - Lys - Ile - Glu - Lys - Phe - Gln - Ser - Glu - Glu - Gln - Gln - Gln -
                                                          (Absent in Variant C)   P     Lys   Lys (Variant C)
                                                                                      (Variant E)
                                                   50                                                               60
Thr - Glu - Asp - Glu - Leu - Gln - Asp - Lys - Ile - His - Pro - Phe - Ala - Gln - Thr - Gln - Ser - Leu - Val - Tyr -
                                                                                                                    80
                               67                  70
Pro - Phe - Pro - Gly - Pro - Ile - Pro - Asn - Ser - Leu - Pro - Gln - Asn - Ile - Pro - Pro - Leu - Thr - Gln - Thr -
       (Variants C, A1, and B)    His
                                                   90                                                              100
Pro - Val - Val - Val - Pro - Pro - Phe - Leu - Gln - Pro - Glu - Val - Met - Gly - Val - Ser - Lys - Val - Lys - Glu -
                                                                                                                   120
                         105 ↓ 106   107 ↓ 108       110
Ala - Met - Ala - Pro - Lys - His - Lys - Glu - Met - Pro - Phe - Pro - Lys - Tyr - Pro - Val - Gln - Pro - Phe - Thr -
                              Gln (Variant A3)
                                                   130                                                             140
       122
Glu - Ser - Gln - Ser - Leu - Thr - Leu - Thr - Asp - Val - Glu - Asn - Leu - His - Leu - Pro - Pro - Leu - Leu - Leu -
      Arg  (Variant B)
                                                   150                                                             160
Gln - Ser - Trp - Met - His - Gln - Pro - His - Gln - Pro - Leu - Pro - Pro - Thr - Val - Met - Phe - Pro - Pro - Gln -
                                                                                                                   180
                                                   170
Ser - Val - Leu - Ser - Leu - Ser - Gln - Ser - Lys - Val - Leu - Pro - Val - Pro - Glu - Lys - Ala - Val - Pro - Tyr -
                                                                                                                   200
                                                   190
Pro - Gln - Arg - Asp - Met - Pro - Ile - Gln - Ala - Phe - Leu - Leu - Tyr - Gln - Gln - Pro - Val - Leu - Gly - Pro -

                                            209
Val - Arg - Gly - Pro - Phe - Pro - Ile - Ile - Val.OH
```

Figure 3. Primary structure of *Bos* β-CN A^2-5P (103, 216). The enclosed amino acid residues are the sites corresponding to the mutational (99, 102, 104) differences in the genetic variants: A^1, A^3, B, C, D, and E. The arrows indicate the points of attack by plasmin responsible for β-CN fragments present in milk.

10 20
PyroGlu - Glu - Gln - Asn - Gln - Glu - Gln - Pro - Ile - Arg - Cys - Glu - Lys - Asp - Glu - Arg - Phe - Phe - Ser - Asp -

30 40
Lys - Ile - Ala - Lys - Tyr - Ile - Pro - Ile - Gln - Tyr - Val - Leu - Ser - Arg - Tyr - Pro - Ser - Tyr - Gly - Leu -

50 60
Asn - Tyr - Tyr - Gln - Gln - Lys - Pro - Val - Ala - Leu - Ile - Asn - Asn - Gln - Phe - Leu - Pro - Tyr - Pro - Tyr -

70 80
Tyr - Ala - Lys - Pro - Ala - Ala - Val - Arg - Ser - Pro - Ala - Gln - Ile - Leu - Gln - Trp - Gln - Val - Leu - Ser -

90 100
Asp - Thr - Val - Pro - Ala - Lys - Ser - Cys - Gln - Ala - Gln - Pro - Thr - Thr - Met - Ala - Arg - His - Pro - His -

105 ↓ 106 110 120
Pro - His - Leu - Ser - Phe - Met - Ala - Ile - Pro - Pro - Lys - Lys - Asn - Gln - Asp - Lys - Thr - Glu - Ile - Pro -

130 136 140
Thr - Ile - Asn - Thr - Ile - Ala - Ser - Gly - Glu - Pro - Thr - Ser - Thr - Pro - Thr - $\boxed{\text{Ile}}$ - Glu - Ala - Val - Glu -
Thr (Variant A)

148 150 160
Ser - Thr - Val - Ala - Thr - Leu - Glu - $\boxed{\text{Ala}}$ - Ser - Pro - Glu - Val - Ile - Glu - Ser - Pro - Pro - Glu - Ile - Asn -
(Variant A) Asp P

169
Thr - Val - Gln - Val - Thr - Ser - Thr - Ala - Val.OH

Figure 4. Primary structure of *Bos* κ-CN B-1P (186). The enclosed amino acid residues are the sites corresponding to the mutational differences in the A variant (186). The arrow indicates the point of attack by chymosin (rennin).

similar reasoning is called β-CN A (f 108-209). As yet, the specific genetic variants of the β-CN fragments (f 1-105), (f 1-107), (f 29-105), and (f 29-107) have not been identified, but their genetic nomenclature is expected to relate in similar manner to the β-CN variant from which they were formed.

κ-Caseins

The κ-casein (κ-CN) family consists of a major carbohydrate-free component and a minimum of six minor components (65, 66, 172, 210, 224, 256, 271, 273). The κ-CN, as isolated from milk, occurs in the form of a mixture of polymers held together by intermolecular disulfide bonds (236). However, Beeby (20) reported free thiol groups after calcium removal by treatment with ethylenediaminetetraacetate (EDTA) or oxalate. He suggested that the native form may be the reduced monomer rather than the disulfide-linked polymers.

The primary structure of the major carbohydrate-free component of κ-CN B-1P (Figure 4) consists of 169 amino acid residues (186): Asp_4, Asn_7, Thr_{14}, Ser_{12}, Ser P_1, $Pyroglu_1$, Glu_{12}, Gln_{14}, Pro_{20}, Gly_2, Ala_{15}, Cys_2, Val_{11}, Met_2, Ile_{13}, Leu_8, Tyr_9, Phe_4, Lys_9, His_3, Trp_1, and Arg_5 with a calculated molecular weight of 19, 007. There is still some question concerning the N-terminal pyroglutamyl residue in the native protein because cyclization may occur during isolation (234).

Two genetic variants, designated A and B, are known (189, 223, 270). The κ-CN A-1P differs from the B variant (Table 2) by substitution of a threonine residue for isoleucine at position 136 and an aspartic acid residue for alanine at position 148 (186). The A variant tends to be predominant in most breeds (11). In alkaline gel electrophoresis in the presence of mercaptoethanol and urea, both variants show multiple bands with the A variant possessing the greater mobility (171, 238).

The bond sensitive to chymosin (EC 3.4.23.4) (rennin) hydrolysis occurs between the phenylalanine residue at position 105 and the methionine residue at position 106 (Figure 4) (64, 131, 166, 204). The hydrolytic products are para-κ-casein (residues 1-105) and the macropeptides (residues 106-169). Doi et al. (65) and Vreeman et al. (256) observed para-κ-casein in purified preparations of κ-CN. This is due to a chymosin-like proteolysis subsequent to translation, but more work must be done before concluding that para-κ-casein is a natural constituent of milk rather than a product of storage or of the preparatory process.

Structures of the minor κ-CN components have not been established, and considerable disagreement exists among investigators. Generally, the minor κ-CN are believed to be glycosylated forms of the major κ-CN, but even this has not been confirmed by all investigators. Vreemen et al. (256) concluded from their investigation that, in order of elution from DEAE-cellulose, the adsorbed κ-CN were the major component free of carbohydrate with one phosphate group; a component containing one phosphate and one carbohydrate moiety with a N-acetylneuraminic acid (NeuNAc) residue; a component containing one phosphate group and one carbohydrate moiety with two NeuNAc residues in a branched configuration; a component with two phosphate groups and no NeuNAc; a component with one phosphate and two carbohydrate moieties with two NeuNAc residues each; a component probably containing two phosphate groups and one carbohydrate moiety with two NeuNAc residues; and a component with either two phosphate and two carbohydrates containing two NeuNAc or one phosphate and three comparable carbohydrate chains. In contrast, Doi et al. (65, 66) concluded from their fractionation of κ-CN on DEAE-cellulose that there are five major and two minor adsorbed components all containing one phosphate group. Of the major components, the first is the major carbohydrate-free component of κ-CN; the second contains 1 mole each of galactose (Gal) and galactosamine (GalNAc) per mole; the third has 1 mole each of Gal, GalNAc, and NueNAc per mole; the fourth has 3 moles of Gal and 2 moles each of GalNAc and NeuNAc; and the fifth has 4 moles of Gal and 3 each of GalNAc and NeuNAc. The two minor components appear to differ slightly in amino acid content and contain one and two carbohydrate moieties, respectively. Each moiety contains 1 mole each of Gal, GalNAc, and NeuNAc.

Several others have investigated the structure of carbohydrate moieties and their point of attachment to the peptide chain (80, 81, 82, 132, 133, 134, 253, 263, 264). Fournet et al. (81) isolated three oligosaccharides from κ-CN and determined the structures for two:

$$1)\ \text{NeuNAc} \xrightarrow{\alpha 2,3} \text{Gal} \xrightarrow{\beta 1,3} \text{GalNAc} \xrightarrow{\beta 1} \text{Thr (132)}$$

$$2)\ \text{NeuNAc} \xrightarrow{\alpha 2,3} \text{Gal} \xrightarrow{\beta 1,3} \underset{\displaystyle\text{GalNAc}}{\overset{\displaystyle\text{NeuNAc}}{\downarrow \alpha 2,6}} \xrightarrow{\beta 1} \text{Thr (132)}$$

So far, Thr's at positions 131, 133, and 135 or 136 (81, 82, 130, 132, 134, 142) have been identified as points of attachment of oligosaccharide chains through 0-glycosidic linkages. If an oligosaccharide is attached to κ-CN A-1P at Thr 136, the B variant could not contain an oligosaccharide at this position because Ile replaces Thr (Figure 4, Table 2).

Other carbohydrates also have been detected in preparations of κ-CN. Wheelock and Sinkinson (263, 264) found D-glucose (Glu) and D-mannose (Man) in samples of κ-CN from which free sugars were removed. They separated the para-κ-casein from the glycomacropeptides after rennin treatment and found that although the NeuNAc was associated almost entirely with the glycomacropeptides, the Man primarily was attached to the para-κ-casein and Gal and GalNAc were in both. More recently para-κ-casein was reported devoid of carbohydrate (81).

The carbohydrate on colostral κ-CN is more complex and variable than in normal milk (82). Colostrum κ-CN contains almost twice as much carbohydrate as well as an additional amino sugar, N-acetylglucosamine (GlcNAc) (67). Two of the oligosaccharide chains that have been characterized have structures identical to those (structures 1 and 2) for κ-CN from milk (220, 221). However, four additional oligosaccharides, which appear to be unique to colostral κ-CN, have been characterized and possess the following structures:

3)
$$\begin{array}{ccccccc} & & & & \text{NeuNAc} & & \\ & & & & \downarrow{\scriptstyle \beta2,6} & & \\ \text{GlcNAc} & \xrightarrow{\beta1,3} & \text{Gal} & \xrightarrow{\beta1,3} & \text{GalNAc} & \longrightarrow & (219)\end{array}$$

4)
$$\begin{array}{ccccc} \text{Gal} & \xrightarrow{\beta1,4} & \text{GlcNAC} & & \\ & & \downarrow{\scriptstyle \beta1,6} & & \\ \text{Gal} & \xrightarrow{\beta1,3} & \text{GalNAc} & \longrightarrow & (220)\end{array}$$

5)
$$\begin{array}{ccccccc} & & \text{Gal} & \xrightarrow{\beta1,4} & \text{GlcNAc} & & \\ & & & & \downarrow{\scriptstyle \beta1,6} & & \\ \text{NeuNAC} & \xrightarrow{\alpha2,3} & \text{Gal} & \xrightarrow{\beta1,3} & \text{GalNAc} & \longrightarrow & (114, 220, 221)\end{array}$$

6)
$$\begin{array}{ccccccc} \text{NeuNAc} & \xrightarrow{\alpha2,3} & \text{Gal} & \xrightarrow{\beta1,4} & \text{GlcNAc} & & \\ & & & & \downarrow{\scriptstyle \beta1,6} & & \\ \text{NeuNAc} & \xrightarrow{\alpha2,3} & \text{Gal} & \xrightarrow{\beta1,3} & \text{GalNAc} & \longrightarrow & (221)\end{array}$$

Only Thr's 131, 133, and 135 have been identified as points of attachment for oligosaccharides bound to colostral κ-CN (67).

Because exact strctures of minor components of κ-CN are not known, we think that nomenclature of these components cannot be precise at this time. We suggest that they temporarily be identified according to the genetic variant of the major nonglycosylated component and that they be numbered consecutively according to their increasing relative electrophoretic mobility in alkaline gels in the presence of mercaptoethanol (273) starting as κ-CN A-1 or κ-CN B-1. More conclusive results are needed before nomenclature of the κ-CN's can be finalized.

WHEY PROTEINS

The term "whey proteins" has been used to describe the group of milk proteins that remain soluble in "milk serum" or whey after precipitation of caseins at pH 4.6 and 20°C. Traditionally, β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, and proteose peptone fractions have been considered to be the major characterized components of this fraction. However, recent findings that proteose peptone components 5, 8-slow, and 8-fast are actually fragments of β-CN (7, 8, 74) and that other heat stable polypeptides are present (197) raise questions concerning the utility of this term. Based on current knowledge, the term "whey protein" should be used only in a general sense to describe milk proteins soluble at pH 4.6 and 20°C. Commercial products termed whey protein isolates or concentrates are obtained from cheese manufacture at higher pH's and will contain intact caseins as well as their proteolytic products such as macropeptides and proteose peptone fraction. Individual families, such as β-lactoglobulin, α-lactalbumin, and serum albumin, should be classified according to homology with the primary sequence of their amino acid chains. Polyacrylamide or starch gel electrophoresis still can be used to characterize and identify individual members of each family (238). The characterization of immunoglobulins, proteins not unique to milk, continues to be based upon immunochemical as well as physical and chemical similarities with human and other mammalian immunoglobulins.

β-Lactoglobulin

β-Lactoglobulin B (β-LG) consists of 162 amino acid residues with the following composition: Asp_{10}, Asn_5, Thr_8, Ser_7, Glu_{16},

Gln_9, Pro_8, Gly_4, Ala_{15}, Cys_5, Val_9, Met_4, Ile_{10}, Leu_{22}, Tyr_4, Phe_4, Lys_{15}, His_2, Trp_2, and Arg_3, and a calculated molecular weight of 18,277. The primary structure (Figure 5) has been determined by Braunitzer and coworkers (35, 36, 85, 86). Several corrections to the original sequence have been made and are included in the primary structure shown for β-LG (Figure 5). Residues 155 and 156 have been changed from leucine-glycine to glycine-leucine (100, 207), and positions 84 and 87 are occupied by isoleucine and leucine instead of leucine and isoleucine (207). However, the proposal that residue 11 is asparagine instead of aspartic acid (100) has not been substantiated (25, 207) and still is shown to be aspartic acid (Figure 5) until additional information becomes available. The one free thiol group that occurs in each molecule of β-Lg appears to be distributed equally between positions 119 and 121 (36, 180, 182). When the thiol occurs at residue 119, a disulfide bridge occurs between residues 106 and 121, and between residues 106 and 119 when the free thiol is at position 121. In either situation, the other disulfide bridge always occurs between positions 66 and 160 (Figure 5).

Seven genetic variants of β-LG are known (12, 24, 25, 40, 100, 199). Positions of amino acid substitutions have been determined for five of these variants, A, B, C, D, and E (Figure 5, Table 2). Yak (*Bos grunniens*) milk contains only a single type of β-LG with an electrophoretic mobility similar to bovine β-LG D (100). However, this β-LG differs from the bovine B variant (Figure 5) by substitution of glycine for glutamic acid at 158 (100) instead of glutamine for glutamic acid at 45 as in the D variant (Table 2). Grosclaude et al. (100) suggested that this variant be labeled β-LG D_{yak}. However, because we wish to avoid subscripts and superscripts, we suggest that this variant in yak milk be called, in order of its discovery, β-LG E (Table 2). Three additional genetic variants of β-LG (E, F, and G) have been observed in the milk of Bali (Banteng) cattle, *Bos* (Bibos) *javanicus* (25). Through a combination of amino acid analysis, tryptic peptide maps, and amino acid analyses of different peptides, Bell et al. (25) showed that β-LG E in Bali milk appears to possess the same glycine/glutamic acid substitution at position 158 as the β-LG D_{yak} we now suggest be called β-LG E. The β-LG F appears to differ from the bovine B variant by this same substitution at 158 as well as substitution of serine for proline at 50 and tyrosine for aspartic acid at either 129 or 130. The β-LG G also appears to differ from

```
                                            10                                                          20
H.Leu - Ile - Val - Thr - Gln - Thr - Met - Lys - Gly - Leu - Asp - Ile - Gln - Lys - Val - Ala - Gly - Thr - Trp - Tyr -

                                            30                                                          40
Ser - Leu - Ala - Met - Ala - Ala - Ser - Asp - Ile - Ser - Leu - Leu - Asp - Ala - Gln - Ser - Ala - Pro - Leu - Arg -

                        45                  50                                                    59    60
Val - Tyr - Val - Glu - Glu - Leu - Lys - Pro - Thr - Pro - Glu - Gly - Asp - Leu - Glu - Ile - Leu - Leu - Gln - Lys -
          (Variant D)  Gln                                                                        His (Variant C)
                  64                        70                                                          80
Trp - Glu - Asn - Gly - Glu - Cys - Ala - Gln - Lys - Lys - Ile - Ile - Ala - Glu - Lys - Thr - Lys - Ile - Pro - Ala -
                  Asp (Variant A)
                                            90                                                          100
Val - Phe - Lys - Ile - Asp - Ala - Leu - Asn - Glu - Asn - Lys - Val - Leu - Val - Leu - Asp - Thr - Asp - Tyr - Lys -

                                            110                                             118   SH    120
Lys - Tyr - Leu - Leu - Phe - Cys - Met - Glu - Asn - Ser - Ala - Glu - Pro - Glu - Gln - Ser - Leu - Ala - Cys - Gln -
                                                                                            Val (Variant A)
SH                                          130                                                         140
Cys - Leu - Val - Arg - Thr - Pro - Glu - Val - Asp - Asp - Glu - Ala - Leu - Glu - Lys - Phe - Asp - Lys - Ala - Leu -

                                            150                                             158         160
Lys - Ala - Leu - Pro - Met - His - Ile - Arg - Leu - Ser - Phe - Asn - Pro - Thr - Gln - Leu - Glu - Glu - Gln - Cys -
                                                                                (Variant E) Gly
      162
His - Ile.OH
```

Figure 5. Primary structure of *Bos* β-LG B (35, 36, 85, 86). The locations of the SH-group and the disulfides are deduced by applying the observations of McKenzie et al (180, 182) to the most recent primary sequence of Braunitzer et al (85, 86). The SH-group is assumed to exist in a 50:50 distribution between positions 119 and 121 with the -SS-bridge location depending upon the position of the SH-group. The enclosed amino acid residues are the sites corresponding to the differences in the genetic variants.

the B variant by substitution of glycine for glutamic acid at position 158 as well as methionine for isoleucine at position 78 (Table 2). However, the exact locations of amino acid substitutions in β-LG F and G still need to be verified by sequencing of the amino acid chains.

A report of this Committee (267) mentioned an additional variant of β-LG occurring rarely in milk of Droughtmaster cattle (24). This variant originally was reported to be identical to the A variant except for the presence of covalently-attached carbohydrate. Carbohydrates present are NeuNAc, GlcNAc, GalNAc, Man, and Gal in the proportions 1.0:3.4:.9:1.9:.8 (24). This glycosylated form should not be considered a genetic variant of β-LG, because it does not appear to occur through point mutation or deletion but rather through posttranslational modification. Recently this variant was reported to differ from β-LG A by substitution of asparagine for aspartic acid at residue 28 (25), the point of attachment for the carbohydrate moiety. If this substitution can be established, then this form of β-LG in Droughtmaster milk is a true genetic variant. In the meantime, we suggest that the term β-LG Dr be retained temporarily until the nature of the variation is established.

α-Lactalbumin

The interaction of α-lactalbumin (α-LA) with galactosyltransferase to produce lactose has been the subject of several reviews (39, 70, 117, 136, 137). The complete amino acid sequence of bovine α-LA has been determined (Figure 6) by Brew et al. (37, 38, 255). As shown, the B variant consists of 123 amino acid residues with the composition: Asp_9, Asn_{12}, Thr_7, Ser_7, Glu_8, Gln_5, Pro_2, Gly_6, Ala_3, Cys_8, Val_6, Met_1, Ile_8, Leu_{13}, Tyr_4, Phe_4, Lys_{12}, His_3, Trp_4, and Arg_1, and a calculated molecular weight of 14,175. All of the half-cystine residues are connected by intramolecular disulfide bonds (Figure 6). Studies involving metal analyses and effects of EDTA on denaturation indicate that α-LA binds calcium (118).

Three genetic variants, A, B, and C, are known. Only the B variant has been observed in milk from Western cattle, whereas both A and B occur in milk from African Fulani and African and Indian Zebu cattle (10, 29). The α-LA A differs from B in the substitution of a glutamine residue for arginine at position 10 (Table 2) (93). The α-LA prepared from milk of Bali cattle migrates more slowly in alkaline gel electrophoresis than bovine α-LA B (23). Although designated as α-LA C (23), the amino acid substitutions that differentiate this variant have not been established.

Minor forms of bovine α-LA have been reported and some of these contain carbohydrates (13, 16, 17, 23, 93, 124). Aschaffenburg and Drewry (13) observed a faster moving component in an α-LA preparation on paper electrophoresis at pH 8.6. This protein had

10 20
H.Glu - Gln - Leu - Thr - Lys - Cys - Glu - Val - Phe - $\boxed{\text{Arg}}$ - Glu - Leu - Lys - Asp - Leu - Lys - Gly - Tyr - Gly - Gly -
Gln (Variant A)

30 40
Val - Ser - Leu - Pro - Glu - Trp - Val - Cys - Thr - Thr - Phe - His - Thr - Ser - Gly - Tyr - Asp - Thr - Glu - Ala -

50 60
Ile - Val - Glu - Asn - Asn - Gln - Ser - Thr - Asp - Tyr - Gly - Leu - Phe - Gln - Ile - Asn - Asn - Lys - Ile - Trp -

70 80
Cys - Lys - Asn - Asp - Gln - Asp - Pro - His - Ser - Ser - Asn - Ile - Cys - Asn - Ile - Ser - Cys - Asp - Lys - Phe -

90 100
Leu - Asn - Asn - Asp - Leu - Thr - Asn - Asn - Ile - Met - Cys - Val - Lys - Lys - Ile - Leu - Asp - Lys - Val - Gly -

110 120
Ile - Asn - Tyr - Trp - Leu - Ala - His - Lys - Ala - Leu - Cys - Ser - Glu - Lys - Leu - Asp - Gln - Trp - Leu - Cys -

123
Glu - Lys - Leu.OH

Figure 6. Primary sequence of *Bos* α-LA B (37, 38, 255). The disulfide bridges in the molecule are as indicated between positions 6 and 120; 28 and 111; 61 and 77; and 73 and 91. The enclosed amino acid residue is the site corresponding to the difference in genetic variant A.

the same amino acid composition as α-LA but contained one additional hexosamine residue per molecule (93, 94). Barman (16) prepared a glycosylated form of α-LA containing Man, Gal, fucose, GlcNAc, GalNAc, and NeuNAc in proportions 4.1:1.4:1.0:3.1:1.1:.64. Hopper and McKenzie (124) examined minor components of α-LA A and B and found one component with faster electrophoretic mobility than α-LA in starch gels at pH 7.7 and two with slower electrophoretic mobilities. The fast component appears to have one less amide group than α-LA. The faster of the two slow components contains NeuNAc, but the slower one does not. Although α-LA possesses four disulfide bonds (255), Barman (17) reported isolation of a minor fraction (5%) containing only three disulfide bonds. All of these minor components still retain their lactose synthetase activity. More information, therefore, is needed before nomenclature of these minor components of α-LA can be established. In the interim, use of Arabic numbers to differentiate by electrophoretic mobility is suggested.

Serum Albumin

Serum albumin (SA) prepared from milk is physically (203) and immunologically (62) identical to blood SA, the major protein of plasma; the protein prepared from blood plasma has been studied extensively. Since the last report of this committee (267), a complete amino acid sequence of bovine SA has been determined (Figure 7) by Brown (43, 44). The original sequence presented by Brown (43) contained a gap of three (43) or four (44) residues extending from 400 to 403; these residues were identified as Gly-Phe-Gln-Asn by Reed and coworkers (214) and are included in the sequence in Figure 7. The sequence presented by Brown (44) assumed lysine at residue 400 as in human SA (22, 184). However, Reed and coworkers (214) found this residue in *Bos* was glycine rather than lysine. *Bos* SA consists of 582 residues with the composition: Asp_{39}, Asn_{12}, Asx_{3}, Thr_{34}, Ser_{28}, Glu_{59}, Gln_{19}, Glx_{1}, Pro_{28}, Gly_{16}, Ala_{46}, Cys_{35}, Val_{36}, Met_{4}, Ile_{14}, Leu_{61}, Tyr_{19}, Phe_{27}, Lys_{59}, His_{17}, Trp_{2}, and Arg_{23} and a calculated molecular weight of 66,267. Serum albumin has 17 intramolecular disulfide bonds and only one free sulfhydryl group, which occurs at residue 34 (Figure 7). The sequence and disulfide bridges shown do not indicate the structural features of loops and domains proposed by Brown (44). The molecule is visualized as having three major domains, each consisting of two large double loops and a small double loop and appears to be approximately in the shape of 3:1 ellipsoid. The N-terminal region is believed to be more compact than the C-terminal region (198). The domains are dissimilar in hydrophobicity, net charge, and ligand binding sites (198). The appearance of several bands in isoelectric focusing patterns indicate that considerable microheterogeneity may exist (232).

Immunoglobulins

Like SA, immunoglobulins are not unique to lacteal secretions. Except for secretory component (SC) and the complex called secretory immunoglobulin A (SIgA), which it forms with immunoglobulin A (IgA), all immunoglobulins in milk are normally in serum, although concentrations of each in colostrum and milk may vary (34, 52, 53, 168, 205). However, because of their extreme heterogeneity, immunoglobulins differ significantly from other serum proteins. Thus, physical and chemical parameters routinely used to characterize and identify most other milk proteins are only of limited value for immunoglobulins. Their identification must be based primarily upon immunochemical criteria and crossreactivity with reference immunoglobulins that are primarily of human origin. A system of nomenclature already has been proposed for the bovine immunoglobulins (1, 2, 60) based upon a system originally proposed for those of human origin by the World Health Organization (61) and revised (191). Our recommendations for nomenclature of the immunoglobulins in bovine milk, as well as their structural components and closely related proteins, continue to be based upon these systems (1, 2, 60, 61, 191).

Of the five classes of immunoglobulins in mammals, four (IgG, IgA, IgM, and IgE) have been identified in bovine milk. All classes of immunoglobulins exist as either polymers or monomers of a basic unit composed of four polypeptide chains. In all species in which immunoglobulins have been studied extensively, the monomeric form consists of two identical heavy (molecular weight from 50,000 to 70,000, depending upon class) and two identical light (molecular weight about 20,000) poly-

10 20
Asp-Thr-His-Lys-Ser-Glu-Ile-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly-Glu-Glu-His-Phe-Lys-
30 40
Gly-Leu-Val-Leu-Ile-Ala-Phe-Ser-Gln-Tyr-Leu-Gln-Gln-Cys-Pro-Phe-Asp-Glu-His-Val-
50 60
Lys-Leu-Val-Asn-Glu-Leu-Thr-Glu-Phe-Ala-Lys-Thr-Cys-Val-Ala-Asp-Glu-Ser-His-Ala -
70 80
Gly-Cys-Glu-Lys-Ser-Leu-His-Thr-Leu-Phe-Gly-Asp-Glu-Leu-Cys-Lys-Val-Ala-Ser-Leu-
90 100
Arg-Glu-Thr-Tyr-Gly-Asp-Met-Ala-Asp-Cys-Cys-Glu-Lys-Glu-Gln-Pro-Glu-Arg-Asn-Glu-
110 120
Cys-Phe-Leu-Ser-His-Lys-Asp-Asp-Ser-Pro-Asp-Leu-Pro-Lys-Leu-Lys-Pro-Asp-Pro-Asn-
130 140
Thr-Leu-Cys-Asp-Glu-Phe-Lys-Ala-Asp-Glu-Lys-Lys-Phe-Trp-Gly-Lys-Tyr-Leu-Tyr-Glu-
150 160
Ile-Ala-Arg-Arg-His-Pro-Tyr-Phe-Tyr-Ala-Pro-Glu-Leu-Leu-Tyr-Ala-Asn-Lys-Tyr-Asn-
170 180
Gly-Val-Phe-Gln-Glu-Cys-Cys-Gln-Ala-Glu-Asp-Lys-Gly-Ala-Cys-Leu-Leu-Pro-Lys-Ile-
190 200
Glu-Thr-Met-Arg-Glu-Lys-Val-Leu-Thr-Ser-Ser-Ala-Arg-Gln-Arg-Leu-Arg-Cys-Ala-Ser-
210 220
Ile-Gln-Lys-Phe-Gly-Glu-Arg-Ala-Leu-Lys-Ala-Trp-Ser-Val-Ala-Arg-Leu-Ser-Gln-Lys-
230 240
Phe-Pro-Lys-Ala-Glu-Phe-Val-Glu-Val-Thr-Lys-Leu-Val-Thr-Asp-Leu-Thr-Lys-Val-His-
250 260
Lys-Glu-Cys-Cys-His-Gly-Asp-Leu-Leu-Glu-Cys-Ala-Asp-Asp-Arg-Ala-Asp-Leu-Ala-Lys-
270 280
Tyr-Ile-Cys-Asx-Asx-Glx-Asx-Thr-Ile-Ser-Ser-Lys-Leu-Lys-Glu-Cys-Lys-Asp-Pro-Cys-
290 300
Leu-Leu-Glu-Lys-Ser-His-Cys-Ile-Ala-Glu-Val-Glu-Lys-Asp-Ala-Ile-Pro-Glu-Asp-Leu-
310 320
Pro-Pro-Leu-Thr-Ala-Asp-Phe-Ala-Glu-Asp-Lys-Asp-Val-Cys-Lys-Asn-Tyr-Gln-Glu-Ala-
330 340
Lys-Asp-Ala-Phe-Leu-Gly-Ser-Phe-Leu-Tyr-Glu-Tyr-Ser-Arg-Arg-His-Pro-Glu-Tyr-Ala-
350 360
Val-Ser-Val-Leu-Leu-Arg-Leu-Ala-Lys-Glu-Tyr-Glu-Ala-Thr-Leu-Glu-Glu-Cys-Cys-Ala-
370 380
Lys-Asp-Asp-Pro-His-Ala-Cys-Tyr-Thr-Ser-Val-Phe-Asp-Lys-Leu-Lys-His-Leu-Val-Asp-
390 400
Glu-Pro-Gln-Asn-Leu-Ile-Lys-Gln-Asn-Cys-Asp-Gln-Phe-Glu-Lys-Leu-Gly-Glu-Tyr-Gly-
410 420
Phe-Gln-Asn-Ala-Leu-Ile-Val-Arg-Tyr-Thr-Arg-Lys-Val-Pro-Gln-Val-Ser-Thr-Pro-Thr-
430 440
Leu-Val-Glu-Val-Ser-Arg-Ser-Leu-Gly-Lys-Val-Gly-Thr-Arg-Cys-Cys-Thr-Lys-Pro-Glu-
450 460
Ser-Glu-Arg-Met-Pro-Cys-Thr-Glu-Asp-Tyr-Leu-Ser-Leu-Ile-Leu-Asn-Arg-Leu-Cys-Val-
470 480
Leu-His-Glu-Lys-Thr-Pro-Val-Ser-Glu-Lys-Val-Thr-Lys-Cys-Cys-Thr-Glu-Ser-Leu-Val-
490 500
Asn-Arg-Arg-Pro-Cys-Phe-Ser-Ala-Leu-Thr-Pro-Asp-Glu-Thr-Tyr-Val-Pro-Lys-Ala-Phe-
510 520
Asp-Glu-Lys-Leu-Phe-Thr-Phe-His-Ala-Asp-Ile-Cys-Thr-Leu-Pro-Asp-Thr-Glu-Lys-Gln-
530 540
Ile-Lys-Lys-Gln-Thr-Ala-Leu-Val-Glu-Leu-Leu-Lys-His-Lys-Pro-Lys-Ala-Thr-Glu-Glu-
550 560
Gln-Leu-Lys-Thr-Val-Met-Glu-Asn-Phe-Val-Ala-Phe-Val-Asp-Lys-Cys-Cys-Ala-Ala-Asp-
570 580
Asp-Lys-Glu-Ala-Cys-Phe-Ala-Val-Glu-Gly-Pro-Lys-Leu-Val-Val-Ser-Thr-Gln-Thr-Ala-
582
Leu-Ala.OH

Figure 7. Primary sequence of *Bos* SA (43, 44). The 17 disulfide bridges are indicated by solid lines; the only free sulfhydryl group occurs as residue 34.

peptide chains that are linked covalently by disulfide bonds. All evidence indicates that basic structures for the various classes of immunoglobulins in *Bos* are similar to those of human origin (49, 50, 55, 148, 155). The N-terminal half (first 107 to 115 amino acid residues) of each pair of identical light polypeptide chains is called the variable region because of its highly variable amino acid sequence. Because the sequence of the C-terminal halves are more similar, they are called the constant region. Each pair of identical heavy polypeptide chains also contains a variable (first 107 to 115 residues depending on the variable region subgroup) and a constant (310 to 500 amino acid residues at C-terminus) region. Variable regions of both heavy and light polypeptide chains are responsible for antigen binding, whereas functions of complement fixation, membrane transport, catabolism, and mediation of immediate-type hypersensitivity all have been attributed to the constant region of the heavy polypeptide chains (90). The lower portion of this region generated by proteolysis (51) has been isolated and crystallized and is termed the crystalline fraction (Fc).

Each class of immunoglobulin differs by the sequence of amino acids in the constant region of their heavy polypeptide chains. These differences can be recognized primarily by use of specific antisera to these class-specific determinants. Greek letters, corresponding to Latin letters used to designate each class of immunoglobulins, are assigned to each pair of heavy polypeptide chains that account for these differences (e.g., γ for heavy chains of IgG) (49, 90). When differences in amino acid sequences and, consequently, antigenic determinants, are more subtle, the difference is one of subclass. Numerical subscripts are used with Greek letters to designate these heavy polypeptide chains (e.g., γ_1 for heavy chains in IgG_1). The term allotype is used to designate genetic variations within species similar to blood group antigens.

Light polypeptide chains also can be differentiated by variations within their constant regions. Two such types of light chains, called κ and λ, occur in all classes of immunoglobulins (90). Although both types of light chains also occur in bovine immunoglobulins, the proportion of λ type light chains is higher (122).

Immunoglobulin G (IgG) is the principal class in bovine colostrum and milk. The IgG exists primarily as a monomer of the basic four polypeptide chain unit consisting of two identical heavy (γ) and two identical light (λ or κ) polypeptide chains. The two major subclasses in milk and colostrum of *Bos* are IgG_1 and IgG_2. These subclasses can be distinguished antigenically by determinants in the Fc portion of the heavy (γ_1 and γ_2) polypeptide chains (78). Common antigenic determinants are also in these same constant regions (209; K. Nielson, personal communication). Even though bovine serum contains nearly equivalent amounts of IgG_1 and IgG_2, colostrum and precolostral secretions typically contain 15 to 20 times as much IgG_1 as IgG_2 (113). After transition from colostrum to milk, IgG_1 levels still remain 4 to 7 times greater than IgG_2 (113). Elevated IgG_2 has been reported in milk during inflammation of the gland, the source of which has been discussed elsewhere (57, 170, 257, 274). Low molecular weight forms of IgG have been found in bovine milk and colostrum (21, 51, 92). Although some have been similar to the $F(ab)_2$, Fab, and Fc fragments, which are generated by proteolysis of IgG with pepsin and papain (51), additional information is needed before their nomenclature can be recommended.

The considerable charge heterogeneity between IgG_1 and IgG_2 provides an excellent example for the necessity of using immunological criteria for identification and characterization of immunoglobulins. Ion-exchange chromatography can be used routinely to obtain relatively pure preparations of IgG_1 and IgG_2. Usually, IgG_2 does not bind or is only weakly bound by DEAE- or QAE-cellulose, whereas IgG_1 is removed through use of salt. However, in some cattle the extreme charge heterogeneity IgG_1 and IgG_2 results in overlap of the chromatographic peaks to such an extent that purification, characterization, or both, based solely upon ion-exchange behavior is virtually impossible (54, 55). Only immunological crossreactivity with authentic bovine IgG_1 and IgG_2 can be used for identification.

The IgA in bovine lacteal secretions exists primarily as a dimer of the basic four polypeptide chain unit (58, 68, 96), although some higher aggregates and some apparent degradation products can be found both in milk and colostrum. The basic polypeptide chain unit of IgA consists of two identical heavy (α) and two identical light (λ or κ) chains. Bovine IgA concentrations are elevated in colostrum with concentrations dropping rapidly throughout lactation. Total output of IgA by the mammary gland appears to remain nearly constant (2.5 g/day) throughout lactation; changes in volume greatly influence actual concentration during various stages of lactation (113).

In addition to existing as a dimer in lacteal secretions, IgA also occurs in a complex called SIgA (59, 152, 167, 212, 247), which consists of 1 mole of dimeric IgA covalently attached to 1 mole each of SC and J-chain. Bovine SC, which was isolated first in free form from milk and called glycoprotein a (50, 56, 108), has a molecular weight of 79,000 calculated from its amino acid content (160). Both IgA and SC have been isolated and characterized (50, 68, 160, 168, 169, 206, 254) and are homologous to human IgA and SC (55, 160, 167). Heterogeneity in the SC fraction may be due in part to varying degrees of glycosylation (160) and in part to occurrence of proteolytic fragments known to form during storage of SC (208). More information concerning the nature of this heterogeneity is needed before nomenclature can be recommended. Bovine J-chain has a molecular weight of 16,500 and contains about 10 thiols/mole.

Immunoglobulin M (IgM), which has a sedimentation constant of 19 s (96, 158), usually exists as a pentamer of the basic four polypeptide chain unit. Each mole of pentameric IgM in milk appears also to contain 1 mole of covalently-bound J-chain as well as about 1 mole of noncovalently attached J-chain (155). Each basic four polypeptide chain unit of IgM consists of two identical heavy (μ) and two identical light (λ or κ) polypeptide chains. Bovine IgM, which has been purified from milk by a variety of methods (79, 251), is antigenically homologous to human IgM (183) as well as similar to its counterparts in other species. The IgM concentration in milk averages about .1 mg/ml (113, 251) and is elevated in colostrum (about 6 mg/ml) with gradual declin throughout lactation. Output of IgM in mid-lactation milk is about 1 g/day, about half that produced during early lactation (113).

A protein that possesses reagenic activity and does not belong to IgG, IgA, or IgM classes is transmitted to suckled calves through colostrum (26, 115). This protein generally has been accepted to be IgE and crossreacts with human IgE (192). The molecular size of this protein falls between IgG and IgM (115, 261), which is consistent with the behavior of IgE in other species. Additional information is needed to characterize more fully this bovine immunoglobulin.

β_2-Microglobulin

β_2-Microglobulin (β_2-m) first was prepared from bovine milk by Groves and coworkers (105, 107) as lactollin. This minor milk protein is mentioned in this report since Groves and Greenberg (111, 112) showed that lactollin is bovine β_2-m. Human β_2-m is part of the histocompatibility complex in man and is thought to be necessary for native conformation and antigenic activity of the latter (162). Human β_2-m shows homology to the constant regions of human IgG heavy and light chains, especially C_H3 (200).

Bovine β_2-m concentrations were higher in colostrum than in milk (105, 107). Measurements by competitive immunoassay revealed concentrations as high as 6.0 μg/ml in colostrum and about 4.0 μg/ml in mature milk (208). The amino acid sequence of bovine β_2-m has been completed (112) and is in Figure 8. Bovine

```
                                                      10                                                20
H.Ile - Gln - Arg - Pro - Pro - Lys - Ile - Gln - Val - Tyr - Ser - Arg - His - Pro - Pro - Glu - Asn - Gly - Lys - Pro -

                                                      30                                                40
  Asn - Tyr - Leu - Asn - Cys - Tyr - Val - Tyr - Gly - Phe - His - Pro - Pro - Gln - Ile - Glu - Ile - Asp - Leu - Leu -

                                                      50                                                60
  Lys - Asn - Gly - Glu - Lys - Ile - Lys - Ser - Glu - Gln - Ser - Asp - Leu - Ser - Phe - Ser - Lys - Asp - Trp - Ser -

                                                      70                                                80
  Phe - Tyr - Leu - Leu - Ser - His - Ala - Glu - Phe - Thr - Pro - Asp - Ser - Lys - Asp - Glu - Tyr - Ser - Cys - Arg -

                                                      90                                   98
  Val - Lys - His - Val - Thr - Leu - Glu - Gln - Pro - Arg - Ile - Val - Lys - Trp - Asp - Arg - Asp - Leu.OH
```

Figure 8. Primary sequence of *Bos* β_2-m determined by Groves and Greenberg (112). β_2-m has been shown to be identical with a minor milk protein previously identified as lactollin (111, 112).

β_2-m consists of 98 amino acid residues with the composition: Asp_7, Asn_4, Thr_2, Ser_9, Glu_7, Gln_5, Pro_9, Gly_3, Ala_1, Cys_2, Val_5, Ile_6, Leu_8, Tyr_6, Phe_4, Lys_9, His_4, Trp_2, and Arg_5 with a calculated molecular weight of 11,636. Several physical properties of β_2-m have been determined recently (159).

PROTEIN OF MILK FAT GLOBULE MEMBRANE

According to King (150), first mention that fat droplets dispersed in milk are surrounded by a thin membrane was by Ascherson in 1840. For over 120 yr considerable controversy existed over both the origin and nature of this membrane (45, 150). The first report published by this Committee in 1956 (129) defined proteins associated with this membrane as:

> a complex of proteins and enzymes including lipoproteins, alkaline phosphatase, and xanthine oxidase adsorbed on the surface of milk fat globules. This material (or at least a fraction thereof) can be isolated by washing cream and churning.

The nomenclature of milk fat globule membrane proteins was not mentioned in subsequent reports of this Committee (48, 218, 246, 267), primarily because these polypeptides had not been characterized sufficiently. However, during the past 10 yr, a more definitive picture of the milk fat globule membrane (MFGM) and its associated polypeptides has evolved through increased knowledge of biological membranes (230) and of the mechanism of synthesis and secretion of milk (4, 146, 147, 196, 269). Fat droplets form intracellularly and become enveloped progressively in apical plasma membrane as they are secreted into the alveolar lumen. Thus, proteins associated with the MFGM are derived in part from the apical plasma membrane and in part from the cytosol through adherence to fat droplets or to the internal side of the plasma membrane (89, 269). Thus, a concise catalogue of all proteins associated with MFGM may not be possible.

In keeping with history, we propose that an operational definition of MFGM be developed until associated proteins can be characterized with respect to biological function or primary sequence of their amino acid chain. This definition must be broad enough to allow for inclusion of new discoveries and observations but, as for skim milk proteins, will not include enumeration of enzyme activities associated with MFGM (4, 45, 196).

Singer (230) proposed use of the terms peripheral or integral to describe the degree of association of proteins with various biological membranes such as MFGM. An integral protein is associated closely with the membrane; that is, it actually may be imbedded in or transverse the membrane and is not washed off easily by a variety of reagents. In contrast, a peripheral protein is associated loosely with the membrane and may be removed easily by washing. The association of α-LA with galactosyltransferase in the Golgi apparatus of lactating mammary gland provides an excellent example. The α-LA is considered a peripheral membrane protein because of its association with galactosyltransferase, whereas the enzyme is an integral component of the Golgi apparatus membrane (230). Although we are dealing with a true biological membrane, our consideration of its proteins is within the context of milk proteins. This rules out peripheral proteins as components of MFGM. Rather, the definition of integral protein appears to suit our needs better at this time. Moreover, most researchers working with MFGM have employed some type of wash to remove skim milk proteins from fat globules (6, 116, 154, 239) and, thus, operationally have excluded α-LA and other peripheral skim milk proteins.

In addition to recommending a method for washing lipid droplets, an operational definition of MFGM proteins also should include history of the milk, method of churning to liberate the membranes, method of harvesting the membranes, and method of separation and characterization of the protein components. Given this operational definition, a provisional nomenclature can be devised to provide better communication among researchers concerned with MFGM proteins.

History of Milk

Because cooling of milk prior to preparation of MFGM leads to changes in morphology (269), crystallization of triglycerides (45), and loss of phospholipids, the use of warm milk has been recommended widely. However, Kobylka and Carraway (154) showed that prior chilling of the milk actually has little effect on overall distribution of major membrane proteins.

In the specific case of MFGM proteins, however, overriding consideration must be given to the presence of proteolytic enzymes such as plasmin. Plasminogen is associated with MFGM preparations (121), and fragments of membrane proteins have been found elsewhere in milk, particularly in the proteose peptone fraction (145). Preparation of MFGM from milks that have aged overnight (5) may lead to generation of artifacts. Thus, the use of fresh, warm, raw milk for the study of MFGM is recommended strongly. However, almost all market milks are subjected to cold storage, and the functional properties, which make dried buttermilk a useful commodity, may be the result of proteolytic action.

Washing of Cream

Although many techniques have been employed to harvest fat globules from milk, centrifugation followed by washing is the most widely used. Fresh warm milk is skimmed conveniently by centrifugation at 5,000 to 10,000 $\times$ *g* for 10 to 20 min at room temperature or higher (153, 154, 178). Large scale preparations generally employ a laboratory separator operated at 40°C (6, 116, 144, 196). In general, cream then is standardized to approximately 30% fat and washed. Deionized water (18, 116, 144), buffered saline (89, 193), and buffered sucrose with (178, 231) or without added Mg^{2+} (6) have been used to wash lipid droplets. Although use of sucrose $\pm$ Mg^{2+} appears to offer some advantage in stabilizing enzymatic activities (4, 45, 239), electrophoretic patterns of MFGM prepared with the various washing media appear to be qualitatively similar (Figure 9). Variations in washing media appear primarily to affect distributions of major membrane proteins. A similar conclusion can be reached by an examination of the literature. Finally, three washes of the fat globules appear to be optimal (179, 239).

Churning

Rupture of fat globules by physical or mechanical means is necessary to liberate membranes. This has been accomplished effectively by freeze thawing (153, 154, 231), blending (6, 18, 178), or by use of a rotating shaker (173, 239). The question of cooling is raised again. It is difficult to churn warm cream effectively. Cooled cream requires less mechanical energy to churn; however, some proteins that are associated loosely with the membrane through hydrophobic interactions could be lost.

Recovery of Membranes

The best method for separating MFGM from buttermilk and fat is by warming the churned material to 37 to 40°C followed by centrifugation of the warmed suspension (6, 154, 178). Crude membrane preparations are pelleted during centrifugation. Times and centrifugal forces vary; however, it appears that standardization at 100,000 $\times$ *g* for 1 h should be recommended. Washing and repelleting can be employed. In large scale operations, butter oil is

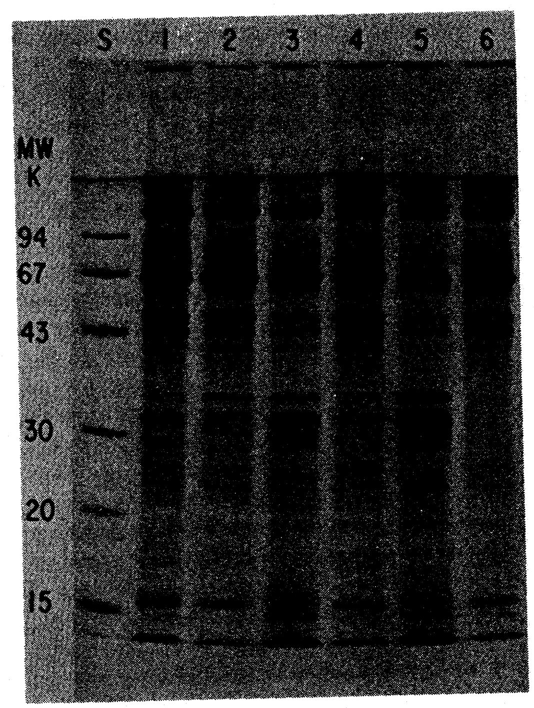

Figure 9. The SDS gel electrophoresis of proteins from milk fat globule membrane (MFGM) prepared by methods of 1) Carraway et al. (153, 231), 2) Anderson et al. (6), 3) Mather and Keenan (178), 4) Nielson and Bjerrum (193), 5) Brunner et al. (45), and 6) Jarasch et al. (125). The gel is a 4 mm slab with 4% and 15% acrylamide in the stacking and separating gels, respectively. The method is essentially that of Laemmli (161). Lane S contains α-LA, trypsin inhibitor, carbonic anhydrase, ovalbumin, SA, and phosphorylase b (bottom to top), which were used as molecular weight markers.

removed by use of a laboratory separator at 40°C, and the membrane may be floated to the top of the buttermilk with $(NH_4)_2SO_4$ (18, 116, 144).

Electrophoretic Separations

The most universally accepted method for characterization of membrane proteins employs electrophoresis in the presence of the detergent sodium dodecyl sulfate (SDS). Thus, the extraction procedure used prior to SDS gel electrophoresis is an integral part of any operational definition for membrane proteins. Although several procedures have been developed for extracting membrane proteins (190, 259, 272), the most successful, and apparently artifact-free, method (201) involves incubation at 37°C for 30 min at alkaline pH with 10% (wt/vol) 2-mercaptoethanol. (Less reductant (272) and shorter incubation times work well with soluble proteins.) Additionally, the extraction medium must contain 1.4 g SDS/g protein, and the concentration of SDS must be at least 1% (wt/vol) (272).

Most workers (18, 151, 173, 178, 229) have used SDS gel electrophoretic procedures to characterize MFGM proteins. These are modifications of the Weber and Osborn (259) continuous buffer gels and give reasonably good resolution of membrane proteins. Anderson et al. (5) employed acrylamide gradient gels, but this method has not been used as widely. Thus far, in all the SDS gel electrophoretic systems attempted, fine division of MFGM proteins has been obtained only in the most carefully done work (178, 229). However, use of a discontinuous gel electrophoretic system, such as that described by Laemmli (161), routinely produces excellent resolution of the fine structure of complex mixtures. This method appears to hold great promise for successful resolution of MFGM proteins as well (19, 177). However, discontinuous gel electrophoresis in SDS must be used with precautions described by Wyckoff et al. (272). These include modifying the Laemmli procedure (161) to eliminate SDS from the gel buffers, reducing SDS in the upper buffer to .03% (wt/vol), and reducing SDS in the total sample load to 150 μg. With these modifications, good resolution of MFGM proteins can be obtained (Figure 9) by the stacking and 10 to 15% acrylamide separating systems of Laemmli (161). This system currently is packaged commercially as Sage by Miles, Inc.[2]

[2] Reference to brand or firm does not constitute endorsement by this committee over others of a similar nature not mentioned.

Definition of Milk Fat Globule Membrane Protein

The Committee recommends the following criteria to serve as an operational definition of MFGM proteins. First, membrane proteins must be associated with the cream fraction obtained from raw uncooled whole milk and remain with the cream after three washings of the fat globules in an aqueous medium. Second, after churning of cream by mechanical means and removal of butter oil at 37 to 40°C, membrane proteins must remain associated with the buttermilk fraction. Third, the protein must coisolate with the membrane fraction prepared from buttermilk either by centrifugation for at least 1 h at 100,000 × g at 4°C or by salting out with 2.2 M $(NH_4)_2SO_4$. Finally, the protein should be extractable with 1% SDS (1.4 g SDS/g protein) and 10% 2-mercaptoethanol and be visualized by staining with either Coomassie blue, periodate acid Schiff reagent or both after SDS gel electrophoresis.

Suggested Nomenclature for Milk Fat Globule Membrane Proteins

When MFGM preparations and molecular weight standards containing α-LA, ovalbumin, and phosphorylase b are examined by the modified Laemmli (161) method described for SDS gel electrophoresis, membrane proteins can be separated into four distinct zones based on their electrophoretic mobilities (Figure 9). Zone A lies between the stacking gel-separating gel interface and phosphorylase b, Zone B between phosphorylase b and ovalbumin, Zone C between ovalbumin and α-LA, and Zone D represents all lower molecular weight proteins that may be true membrane proteins or may be proteolytic products that are not resolved readily and migrate at the "front" of the 15% gels. The majority of proteins associated with MFGM fall into Zones A and B described previously. Because higher percent acrylamide gels require special care, the Committee recommends that the standard gel contain 15% acrylamide. However, because elucidation of Zone A proteins may require lowered content of acrylamide in gels, the nomenclature will allow designation of the percent acrylamide in gels used to characterize the membrane proteins. Changes in percent acrylamide of gels may allow bands to cross over because glycoproteins, in particular, provide anomalous results (5). This situation may be dealt with as the need arises.

The second notation to be used in describing a MFGM protein is the "apparent" molecular weight as interpolated by comparison with standard gels containing molecular weight markers in addition to α-LA, ovalbumin, and phosphorylase b. Finally, the third portion of the nomenclature describes whether the protein stains with Coomassie blue (C), periodate acid Schiff (S), or both after separation by gel electrophoresis. The complete description of a hypothetical protein is:

$$\text{MFGM-A}_{15}\text{-127, C, S}$$

where MFGM indicates that the protein satisfied the operational definition for a MFGM protein, A_{15} designates the zone in a 15% Laemmli (161) gel, 127 is the "apparent" molecular weight in k-daltons, and C, S designates that the protein is both Comassie blue and periodic acid Schiff positive. We recognize the prerogative of the investigators who first describe a protein in detail to provide a trivial name, but we suggest that the nomenclature described be observed as well. One such trivial name that recently has been suggested is butryophillin (87).

NOMENCLATURE OF MILK PROTEINS OF SPECIES OTHER THAN THOSE OF THE GENUS *BOS*

Gel electrophoresis, both in the presence and absence of SDS, has been used to characterize proteins in milks of several species that do not belong to the genus *Bos*. In most cases, the gel electrophoretic patterns are reasonably complex and indicate that milks of these species contain numerous distinct polypeptides. Furthermore, gel electrophoretic techniques often are used to monitor isolation of these milk proteins and to determine purity of a particular preparation. In either situation, a temptation exists to assign names to bands in the electrophoretic patterns obtained from these milks, or to polypeptides actually purified from them, based upon similarities of their electrophoretic mobilities to proteins in bovine milk. However, electrophoretic methods are not good indicators of interspecies protein homology. Classification of a milk protein as a homolog of one of those in *Bos* on the basis of electrophoretic similarity may produce errors. Instead, in this section, we recommend a system of nomenclature to be used for naming proteins obtained from milks of species not belonging to the genus *Bos*. Although this represents a major deviation from the scope of previous reports, we think it is justified by the past tendency of investigators to base nomenclature of milk proteins of other species on that already established for bovine milk.

Nomenclature of Species

We recommend that at first mention of a milk protein in a publication, the mammalian species from which it was obtained be designated by its Latin binomial and vernacular names. Thereafter in the publication either Latin or vernacular nomenclature for the species may be used. A check list of Latin binomial and English vernacular names for mammals is available (123).

Nomenclature of Polypeptide Chain Families

Because species and subspecies within the genus *Bos* can interbreed freely, each of their families of milk proteins can be considered a common pool of genetic polymorphs just as though they were intraspecies variants. Thus far, some milk proteins of Indian cattle (*Bos indicus*), yak (*Bos grunninens*), and Bali cattle (*Bos banteng*) have been isolated and characterized and are included in the tabulation of bovine variants (Table 1). Proteins that may be isolated from milks of other species of the genus *Bos* and characterized should be treated in similar fashion.

We recommend that milk proteins obtained from species not belonging to the genus *Bos* be named, as far as possible, as homologs of the families of proteins known to occur in milk of *Bos*. This recommendation is consistent with the current tendency to name milk proteins from these other species after their bovine counterparts. Names of the principal families of proteins in the milk of *Bos* have been given along with suggested abbreviations (Table 1). Each name, some containing Greek letter prefixes, designates a family of homologous nascent polypeptide chains. Milks of other species contain recognizable homologs of many, but not necessarily all, proteins in bovine milk. For example, milks of humans, guinea pigs, rats, and mice do not contain β-LG (126). However, milks of some other species contain significant amounts of proteins such as lactoferrin, transferrin, and lysozyme, which occur only in extremely low concentrations in bovine milk. Additionally, rat and mouse milks contain, respectively, a group of phosphoproteins, designated P proteins (181), and a lipoprotein, called whey acidic protein (202), for which corresponding homologs have not been identified in bovine milk. When this situation occurs, assignment of nomenclature is left to the discretion of the investigator who is responsible for establishing the uniqueness of that particular milk protein.

Some may question whether milk proteins obtained from either of the two extant species of bison (*Bison bison* and *Bison bonasus*) should be treated as genetic variants or species homologs of bovine milk proteins. Although species belonging to *Bos* and *Bison* interbreed, not all crossbred progeny are fertile. Thus, for the present, we recommend that bison milk proteins be considered homologs, rather than genetic variants, of proteins in bovine milk. To our knowledge, no bison milk proteins have been isolated and characterized. Certainly proteins in the milks of Asiatic and African buffalo (*Bubalus* and *Synceros*) should not be considered genetic variants of *Bos* proteins as members of these two genera do not interbreed with any species belonging to *Bos*.

Nomenclature Assignment

The major difficulty in assigning nomenclature to proteins obtained from milks of other species is in determining their homology with bovine milk proteins. In biological terminology, homology implies divergence from a common ancestor. Although homology can be inferred from similarity in biological function, this characteristic alone should not be considered a safe indicator, as it could have arisen by convergence from different ancestral lines. Although homology also can be inferred through immunological crossreactivity, the most definitive criterion is congruence of amino acid sequence (268).

When a new protein first is isolated from milk of another species, we recommend that it be assigned a provisional name until its homology with a bovine milk protein can be established. If the protein is clearly a casein or whey protein, it can be designated so in the provisional nomenclature. If the protein does not fall clearly into either of these categories, it simply can be called "milk protein". Roman numerals (which are not used anywhere in the permanent nomenclature described in this paper) can be used to designate any fractions that result from application of electrophoresis, gel filtration, or ion-exchange chromatography. Examples of this provisional nomenclature would be domestic cat (*Felis catus*) casein II, raccoon (*Procyon lotor*) whey protein IV, and golden hamster (*Mesocricetus auratus*) milk protein V.

Because the amino acid composition of a protein is usually available long before the primary sequence of the amino acid chain, the ability to determine the existence of homology between two proteins through comparison of their amino acid compositions would be advantageous. The SΔQ characteristic suggested by Marchalonis and Weltman (176) is suitable for this purpose:

$$S\Delta Q = \sum_j (X_{ij} - X_{kj})^2$$

where i and k identify proteins being compared and X_j is the content in moles/100 moles of the amino acid j. If two proteins have SΔQ less than 50, we recommend that they be considered homologous until that time at which their primary sequences can be determined and compared. Homologous protein pairs generally have SΔQ well below 100 (176). If the protein pairs share common biological function as well as SΔQ below 50, homology can be assigned with more certainty. However, similarity in primary sequence of the polypeptide chains still must be retained as the final test for homology between two proteins. In general, two proteins can be considered homologous if their total primary sequences do not differ by more than 50% (63) or if their N-terminal sequences of 20 amino acids do not differ by more than 5 substitutions.

We recommend that genetic polymorphs of each homologous protein within species be designated by placing capital Latin letters after the family name as is currently the practice for bovine milk proteins. Latin letters should be used only to designate genetic variants that have been shown to differ in the substitution of one or more amino acids in the nascent polypeptide chain. These letters should not be used to denote differences in electrophoretic mobilities that might just as easily be due to differences in posttranslational modification. Furthermore, situations such as have occurred with bovine β-CN's A^1, A^2, and A^3 should be avoided. As described, these three genetic variants migrate as a single band in alkaline gels but are resolved as three bands in acid gels. We recommend that no subscripts or superscripts be used. Latin designators should be assigned in chronological order of discovery and characterization of the genetic variant. If only a single genetic variant of a protein is found initially, it immediately should be assigned the designator "A" to preclude possible confusion in the literature when additional variants are discovered.

Posttranslational Modification

We recommend placement of an Arabic number after the Latin designator to differentiate between polypeptide chains that differ as a result of varying degrees of posttranslational phosphorylation, glycosylation, or deamination. As suggested for the various glycosylated and phosphorylated forms of bovine κ-CN, proteins should be numbered consecutively in order of electrophoretic mobility in alkaline gels. When the degree of phosphorylation is known, the Arabic number, along with the letter P, will be used to indicate the actual number of phosphate moieties attached to each polypeptide chain. Thus, two goat caseins that contain 6 and 5 phosphates per molecule and previously were called β_1- and β_2-caseins (217) now would be designated goat (*Capra hircus*) β-CN A-6P and goat (*Capra hircus*) β-CN A-5P, respectively.

We also recommend that proteins present as a result of proteolytic cleavage in milks of other species be named as fragments of the parent polypeptide from which they are derived. The fragment will be designated by enclosing the sequence position number of the first and last amino acid residue of the fragment in parentheses preceded by the letter "f". Thus far, only bovine milk definitely has been shown to contain proteins that are proteolytic derivatives. For example, as discussed, γ_1-casein A^2 is in bovine milk as a result of hydrolysis of β-CN A^2 at Lys 28 by plasmin. Thus, the nomenclature of γ_1-casein A^2 has been changed to β-CN A^2-1P (f 29-209). Proteolytic fragments in milks of other species should be named in similar fashion when sufficient information is available.

Characteristics of proteins isolated from milks of species other than *Bos* have been summarized recently (127, 128).

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